

In the claims

1. (original) Isolated DNA coding for the *MmeI* restriction enzyme, wherein the isolated DNA is obtainable from *Methylophilus methylotrophus*.
2. (currently amended) A recombinant DNA vector comprising a vector into which a DNA segment coding for the *MmeI* restriction enzyme has been inserted.
3. (currently amended) Isolated DNA coding for the *MmeI* endonuclease and ~~*MmeI*~~/methyltransferase, wherein the isolated DNA is obtainable from ATCC Accession No. PTA-4521.
4. (currently amended) A cloning ~~Vectors~~ vector that comprises the isolated DNA of claim 3.
5. (original) A host cell transformed by the vector of claim 2 or 4.
6. (original) A method of producing recombinant *MmeI* restriction endonuclease and *MmeI* methylase comprising culturing a host cell transformed with the vector of claims 2 or 4 under conditions suitable for expression of said endonuclease and methylase.
7. (withdrawn) Isolated DNA coding for an *MmeI*-like restriction enzyme, wherein said isolated DNA hybridizes to at least one conserved motif of the nucleotide sequence coding for the *MmeI* restriction enzyme under predetermined conditions.

Please amend the paragraph beginning on line 7, page 1 as follows:

The present invention relates to a DNA (deoxyribonucleic acid) fragment, which fragment codes for one polypeptide possessing two related enzymatic functions, namely an enzyme which recognizes the DNA sequence 5'-TCC(Pu)AC-3' and cleaves the phosphodiester bond between the 20th and 21st residues 3' to this recognition sequence on this DNA strand, and between the 18th and 19th residues 5' to the recognition sequence on the complement strand 5'-**AGGT(Py)GGTG**-3' to produce a 2 base 3' extension (hereinafter referred to as the MmEI restriction endonuclease), and a second enzymatic activity that recognizes the same DNA sequence, 5'-TCC(Pu)AC-3', but modifies this sequence by the addition of a methyl group to prevent cleavage by the MmEI endonuclease. The present invention also relates to a vector containing the DNA fragment, a transformed host containing this DNA fragment, and an improved process for producing MmEI restriction endonuclease from such a transformed host. The present invention also relates to a process for identifying additional DNA fragments that encode enzymes having the same general properties as MmEI but potentially having unique DNA recognition sequences. This process depends on the use of the amino acid sequence of the MmEI enzyme presented in this application, or subsequently on the additional sequences identified through this process. The invention also relates to additional DNA fragments, identifiable through the process described, each of which

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encodes a polypeptide having significant amino acid sequence similarity to the MmeI polypeptide. The polypeptides encoded by these DNA fragments are predicted to perform similar functions to MmeI. Specifically, they are predicted to possess the dual enzymatic functions of cleaving DNA in a specific manner at a relatively far distance from the specific recognition sequence and also modifying their recognition sequences to protect the host DNA from cleavage by endonuclease activity. An example of such an enzyme identified by this process is CstMI (see U.S. Application Serial No.: 10/616,689, filed concurrently herewith). CstMI was identified as a potential endonuclease because of its highly significant amino acid sequence similarity to MmeI. CstMI recognizes the sequence 5'-AAGGAG-3' and cleaves the phosphodiester bond between the 20th and 21st residues 3' to the recognition sequence on this DNA strand, and between the 18th and 19th residues 5' to the recognition sequence on the complement strand 5'-CTCCTT-3' to produce a 2 base 3' extension.

Please amend the paragraph beginning on line 26, page 7 as follows:

In accordance with the present invention, there is provided a novel DNA fragment encoding a novel restriction endonuclease, obtainable from *Methylophilus methylotrophus* (NEB#1190). The endonuclease is hereinafter referred to as "MmeI", which endonuclease:

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- (1) recognizes the degenerate nucleotide sequence 5'-TCC(Pu)AC-3' in a double-stranded DNA molecule as shown below:

5'-TCC(Pu)AC-3'

3'-AGG(Py)TG-5'

(wherein G represents guanine, C represents cytosine, A represents adenine, T represents thymine, (Pu) represents a purine, either A or G, and (Py) represents a pyrimidine, either C or T);

- (2) cleaves DNA in the phosphodiester bond following the 20th nucleotide 3' to the recognition sequence 5'-TCC(Pu)AC-3 and preceding the 18th nucleotide 5' to the complement strand of the recognition sequence 5'-AGGT(Py)TGCA-3' to produce a 2 base 3' extension:

5'-TCC(Pu)AC(N20)/-3'

3'-AGG(Py)TGT(N18)/-5'; and

- (3) methylates the recognition sequence specified in (1) *in vivo* to protect the host DNA from cleavage by the MmeI endonuclease activity₊.

Please amend the paragraph beginning on line 13, page 10 as follows:

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Figure 1 - Agarose gel showing MmeI cleavage of lambda, T7, phiX174, pBR322 and pUC19 DNAs.

Lane 1: DNA size standards: lambda-HindIII, PhiX174-HaeIII

Lane 2: lambda DNA + MmeI

Lane 3: phage T7 DNA + MmeI

Lane 4: PhiX174 DNA + MmeI

Lane 5: pBR322 DNA + MmeI

Lane 6: pUC19 DNA + MmeI

Lane 7: DNA size standards: lambda-HindIII, PhiX174-HaeIII

Please amend the paragraph beginning on line 22, page 10 as follows:

Figure 4 - Agarose gel showing MmeI cleavage of pTBMmeI.1 DNA and unmodified DNA substrates.

Lane 1: DNA size standards: lambda-HindIII, PhiX174-HaeIII

Lane 2: pTBMmeI.1 (not cut)

Lane 3: pTBMmeI.1 digested with 2.5 units MmeI

Lane 4: lane 3 plus 0.5 µg pRRS vector DNA

Lane 5: lane 3 plus 0.5 µg pRRS PhiX174 DNA

Lane 6: DNA size standards: lambda-HindIII, PhiX174-HaeIII

Lane 7: PhiX174 DNA digested with 2.5 units MmeI

Please amend the paragraph beginning on line 25, page 10 as follows:

Figure 5 - Agarose gel showing MmeI cleavage of unmethylated, hemi-methylated and fully methylated DNA substrates.

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Lane 1: DNA size standards: lambda-BstEII, pBR322-MspI

Lane 2: Unmethylated DNA (oligo1+oligo2) uncut

Lane 3: Unmethylated DNA (oligo1+oligo2) + 2.5 units

MmeI

Lane 4: Unmethylated DNA (oligo1+oligo2) + 2.5 units

Hpy188I

Lane 5: Top methylated/bottom unmethylated DNA
(oligo3+oligo2) uncut

Lane 6: Top methylated/bottom unmethylated DNA
(oligo3+oligo2) + MmeI

Lane 7: Top methylated/ bottom unmethylated DNA
(oligo3+oligo2) + Hpy188I

Lane 8: Top unmethylated/ bottom methylated DNA
(oligo2+oligo4) uncut

Lane 9: Top unmethylated/ bottom methylated DNA
(oligo2+oligo4) + MmeI

Lane 10: Top unmethylated/ bottom methylated DNA
(oligo2+oligo4) + Hpy188I

Lane 11: Methylated DNA (oligo3+oligo4) uncut

Lane 12: Methylated DNA (oligo3+oligo4) + MmeI

Lane 13: Methylated DNA (oligo3+oligo4) + Hpy188I

Lane 14: DNA size standards: lambda-BstEII, pBR322-MspI

Please amend the paragraph beginning on line 16, page 10 as follows:

Figures 2 2A-2E – DNA sequence of the MmeI gene locus
~~(SEQ ID NO:1)~~.

Please amend the paragraph beginning on line 19, page 10 as follows:

Figure 3 – Amino acid sequence of the MmeI gene locus
~~(SEQ ID NO:2)~~.

Please amend the paragraph beginning on line 33, page 10 as follows:

Figures 7 7A-7G – Multiple sequence alignment of MmeI amino acid sequence (SEQ ID NO:3 through SEQ ID NO:14) and homologous polypeptides from public databases.

Please amend the paragraph beginning on line 28, page 35 as follows:

The final assembled sequence (Figures 2A-2E) contained the entire MmeI restriction gene, as well as 1640 bp of sequence preceding the gene and 1610 bp of sequence following the gene.

Please amend the paragraph beginning on line 14, page 32 as follows:

The same group (Tucholski, Gene 223: 293-302 (1998), and Anna Podhajska, personal communication) had previously reported an amino acid sequence of eight residues for a single internal CnBr digestion fragment (sequence GRGRGVGV (SEQ ID NO:50)). PCR based on this sequence was attempted yet failed repeatedly. This sequence was found to be unrelated to MmeI once the actual MmeI amino acid sequence was determined in accordance with the present invention. Therefore correct internal amino acid sequences determination, which enabled the cloning of the MmeI gene, depended on the novel purification method described in this application for the production of sufficiently pure MmeI in large enough quantity to determine cyanogen bromide internal fragment amino acid sequences, as performed in this Application.

Please amend the paragraph beginning on line 21, page 30 as follows:

An example of such an enzyme identified by this process is CstMI (see U.S. Application Serial No. 10/616,689, filed concurrently herewith). CstMI was identified as a potential endonuclease because of its highly significant amino acid sequence similarity to MmeI. CstMI is encoded by sequence #2 above (SEQ ID NO:8), which gave highly significant Expectation value of e^{-171} when compared to MmeI by BLAST. CstMI recognizes the 6 base pair asymmetric sequence 5'-

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AAGGAG-3' and cleaves the DNA in the same manner as MmeI: it cleaves the phosphodiester bond between the 20th and 21st residues 3' to this recognition sequence on this DNA strand, and between the 18th and 19th residues 5' to the recognition sequence on the complement strand 5'-CTCCTT-3' to produce a 2 base 3' extension.